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TITLE: ELECTRICAL RESISTIVITY SENSOR AND SENSING METHOD

INVENTOR: Tsutomu NAGAOKA
Hiroshi SHIIGI

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ATTORNEY: David A. Tucker
(Reg. No.) 27,840
EDWARDS ANGELL PALMER & DODGE LLP
P. O. Box 55874
Boston, Massachusetts 02205
Tel: (617) 517-558
Direct Fax: (617) 888.325.9540

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DESCRIPTION

Electrical Resistivity Sensor and Sensing Method

TECHNICAL FIELD

The present invention relates to an electric resistance type detecting sensor and a detecting method for detecting and verifying a target substance including nucleic acids such as DNA and RNA and proteins such as antigens and antibodies.

BACKGROUND ART

Recently, various kinds of DNA chips have been disclosed in Japanese Unexamined Patent Publication No. 2003-287538 (Patent Document 1), Japanese Unexamined Patent Publication No. 2003-250088 (Patent Document 2) and such. In case of the conventional DNA chips, for example, a sample of a single-stranded DNA is labeled with a fluorescent substance. The sample is then applied to a chip on the surface of which a target DNA being bound, and the target DNA in the sample is allowed to hybridize the DNA which is bound to the surface of the chip. The fluorescent material which has labeled the sample is allowed to emit light. The emitted light is read by means of a microscope or a laser fluorescent scanner so as to detect the presence of the target DNA. In such a technique, however, it cannot be ignored that the DNA is non-specifically absorbed on the substrate, and further, the method of fixing an oligonucleotide probe onto the substrate has not completely established yet. Moreover, the method of controlling the

quantity of the probe to be fixed on the substrate is not sufficiently established. In addition, in order to detect the presence of DNA in the sample, an appropriate fluorescent labeling agent, an intercalater or the like as well as a device such as a laser fluorescent scanner is required. Accordingly, the detection costs high and an operation thereof is troublesome.

In view of the above, Japanese Unexamined Patent Publication No. 2003-514224 (Patent Document 3) discloses a method of detecting the presence of a target DNA by using the change in refractive index of light caused by the surface plasmon resonance (SPR) occurring on a surface of a chip.

Furthermore, Japanese Unexamined Patent Publication No. 2002-533698 (Patent Document 4) discloses a DNA chip having an area where the DNA complementary to a target DNA is bound on a substrate. In this Patent Publication, DNA in a sample is first modified with electroconductive particles and the obtained sample is then applied to the said area to hybridize the DNA bound to the area with the DNA complementary to the above DNA existing in the sample. As a result, when the hybridized DNA exists, an electric current passes through the area via the electroconductive particles which modify the DNA. This is used for detecting the presence of the target DNA.

Therefore, in the above method, it is required that complementary binding partners (6) are necessarily fixed to electroconductive particles (62) while specific binding partners (5) are fixed between two electrodes on the substrate. The fixing of (6) to the

electroconductive particles is achieved by means of sulfhydryl-derivatized oligonucleotides. On the other hand, the fixing of (5) to the substrate is achieved by the silane coating technique which employs APTES, which fixes oligonucleotides as a linker.

In such a detecting method, an electron transfer mediator (an oxidation-reduction or conductive material) is necessarily to be added in order to improve the conductivity between two electrodes. Therefore, a target substance (or a probe) is necessarily to be fixed to an electroconductive particle, deposited between electrodes by means of the probe (or the target substance) fixed to the substrate in advance and detected after amplifying the electric conductivity by adding the mediator or the like.

Accordingly, the above-mentioned detecting method requires the modification of the sample with electroconductive particles, thus the detection is troublesome and costs high.

DISCLOSURE OF THE INVENTION

In view of the above, the present invention is to provide an electric resistance type detecting sensor and an electric resistance type detecting method, which are capable of solving the above problems, namely, capable of electrically detecting and verifying a target substance including nucleic acids such as DNA and RNA and proteins such as antigen and antibody more easily, at lower cost and more accurately than the conventional arts, and further capable of being repeatedly used in an easy way at a low cost.

Accordingly, in view of the first aspect of the invention,

an electric resistance type detecting sensor characterized in that a pair of electrodes is provided oppositely to each other on the surface of an electrically insulated substrate, and a film of electroconductive fine particles modified with a probe is formed on and/or between the electrodes

is provided.

Further, in view of the second aspect of the invention,

an electric resistance type detecting sensor characterized in that a recess is provided on the surface of an electrically insulated substrate, a pair of electrodes is provided oppositely to each other on the recess and a film of electroconductive fine particles modified with a probe is formed on and/or between the electrodes

is provided.

Moreover, in view of the third aspect of the invention,

an electric resistance type detecting sensor characterized in that it comprises a substrate having two or more fine recesses formed on the surface thereof; a film of electroconductive fine particles formed on the inner surface of the respective recesses; and first and second electrodes formed so as to be electrically connected to the film of electroconductive fine particles,

wherein the film of electroconductive fine particles is modified with a probe

is provided.

In addition, in view of the fourth aspect of the invention,

an electric resistance type detecting sensor characterized in that it comprises a substrate having two or more fine recesses formed on the surface thereof; a film of electroconductive fine particles formed on the inner surface of the respective recesses; and first and second electrodes formed so as to be electrically connected to the film of electroconductive fine particles,

wherein the first electrodes are formed on the surface of the substrate and the second electrodes are formed on the inside of the recesses and

the film of electroconductive fine particles is modified with a probe

is provided.

Furthermore, in view of the fifth aspect of the invention,

an electric resistance type detecting method of detecting the presence of a target substance which reacts with a probe, comprising:

modifying, with the probe, a film of electroconductive fine particles formed on the surface of an electrically insulated substrate;

applying a test sample including a substance to be detected to the modified film; and

measuring an electric resistance value between two points of the film of electroconductive fine particles

is provided.

Moreover, in view of the sixth aspect of the invention,

an electric resistance type detecting method of detecting the presence of a target substance which reacts with a probe, comprising:

preparing, in advance, a test sample containing a substance to be detected and the probe;

applying the test sample onto a film of electroconductive fine particles formed on the surface of an electrically insulated substrate; and

measuring an electric resistance value between two points of the film of electroconductive fine particles

is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the selectivity of the present electric resistance type detecting sensor when a single-stranded DNA is used as a probe. Arrows indicate the points of time when the target substance is added dropwise.

Fig. 2 illustrates a variation in electric resistance value of a gold nano-particle film modified with DNA (probe) before and after the DNA existing on the film is degraded with DNase, for the purpose of exemplifying the invention. Arrows indicate the points of time when the target substance and the DNase are added dropwise at the points indicated as 1 and 2, respectively.

Fig. 3 illustrates a variation in electric resistance value of the present electric resistance type detecting sensor in which the single-stranded DNA both ends of which being thiol-derivatized is used as a probe. The arrow shows a point of time when the target substance is added dropwise.

Fig. 4 illustrates a variation in electric resistance value of the present electric resistance type detecting sensor in which the single-stranded DNA both ends of which being thiol-derivatized is used as a probe and a film of electroconductive fine particles does not include any binder. The arrow shows a point of time when the target substance is added dropwise.

Fig. 5 illustrates a variation in electric resistance value of the present electric resistance type detecting sensor in which the single-stranded DNA both ends of which being thiol-derivatized is used as a probe and a test sample are prepared in advance and applied to a film which consists of electroconductive fine particles containing a binder.

Fig. 6 illustrates a variation in resistance value of the gold nano-particle film in which a rabbit-anti-mouse IgG antibody is used as a probe and an antigen of a mouse IgG is used as a target substance, for the purpose of exemplifying the invention. The target substance is added dropwise at the point shown by the arrow.

Fig. 7(a) is a block diagram showing the structure of the electric resistance type detecting sensor in accordance with Example 6 of the present invention. Fig. 7(b) is a side sectional view of the integral part

showing a recess of the electric resistance type detecting sensor.

Fig. 8 is a block diagram showing a structure of the detecting sensor in accordance with Example 6 of the invention.

Fig. 9 is a block diagram showing a structure of the lock-in amplifier circuit in accordance with Example 6 of the invention.

Fig. 10(a) is a block diagram showing a structure of the detecting sensor in accordance with Example 7 of the invention. Fig. 10(b) is a side sectional view of an integral part showing a recess of the detecting sensor.

BEST MODE FOR CARRYING OUT THE INVENTION

In the electric resistance type detecting sensor according to the first embodiment of the invention, a pair of electrodes is provided on the surface of a substrate.

As the "substrate" according to the invention, the substrate which is electrically insulated can be preferably used. The material thereof may specifically include glass, plastics, crystal or silicone.

As a material used for the "electrode" according to the invention, the materials used for an electrode of a conventional sensor is satisfactory. The materials may include metals such as Au, Pt, Cu, Al, Ni and Ti and alloys thereof as well as polymers such as polypyrrole, polyaniline and polyacen. The shape of the electrode is not specifically limited and includes a comb-shaped electrode consisting in the shape of a comb, for example. The electrode may be coated with an insulating material.

On and/or between the electrodes, formed is a film of electroconductive fine particles.

The "film of electroconductive fine particles" according to the invention includes not only the film in which the electroconductive fine particles are arranged to be in direct contact with the electrodes but also the film in a nano-gap state where the electroconductive fine particles are adjacent to the electrodes to the extent that the particles and the electrodes are able to be electrically conductive. From a certain point of view, the film of electroconductive fine particles may be regarded as a layer of electroconductive fine particles.

In the case that the film of electroconductive fine particles mentioned below contains a binder, it is satisfactory that the electroconductive fine particles and the electrodes can be electrically conductive through the binder.

Further, the "electroconductive fine particles" according to the invention include the substance which has an electric conductivity and can directly and/or indirectly bind to the probe mentioned below. The fine particles include particles formed from a material such as carbon, fullerene, platinum, aluminum, gold and silver. Among the above, particles made of metal such as gold and silver are preferred, and particles made of gold are more preferred.

Moreover, the size of the electroconductive fine particles may be properly selected in accordance with a material of the particles or the probe. The average particle diameter of the electroconductive fine particles is preferably a nano-size, further more preferably 50 to 100 nm.

The film of electroconductive fine particles can be formed according to the well known method. For example, in case of using gold nano-particles as the electroconductive fine particles, a film of the gold nano-particles can be formed by contacting with a substrate a gold colloidal solution in which the gold nano-particles are suspended in an appropriate solvent. In this case, water or alcohols such as methanol and ethanol can be used as the solvent.

The film of electroconductive fine particles preferably contains the binder.

The "binder" can be selected appropriately in accordance with the type of the electroconductive fine particles or the probe. Specifically, in case of using metals such as gold or silver as a material for the electroconductive fine particles, the binder may include dithiols having SH group such as 1,10-decanedithiol and diamines having NH_2 group such as 1,10-diaminodecane. Among these, dithiols are preferred, and 1,10-decandithiol is especially preferred.

The film of electroconductive fine particles containing the binder can be formed by the well-known method. In the case that gold nano-particles are used as the electroconductive fine particles and the above-mentioned dithiols or diamines are used as the binder, for example, the film of the gold nano-particles including the binder can be formed by suspending the binder and the gold nano-particles in an appropriate solvent and contacting the thus-prepared suspension with the substrate. In this case, water or alcohols such as methanol and ethanol can be used as the solvent, similar to the above.

The film of electroconductive fine particles including any/no binder according to one embodiment of the invention is modified with the probe.

"To modify the film of electroconductive fine particles of the invention with the probe" includes not only that at least a part of the probe is rendered to be in direct contact with the film of electroconductive fine particles but also that a state of nano-gap is achieved in which the probe and the film of electroconductive fine particles are adjacent enough to the extent that they can be electrically conductive.

In the case that the probe mentioned below is modified with a specific group, the probe may modify the film of electroconductive fine particles via this group.

The "probe" according to the invention includes the probe which can change an electric resistant value of the film of electroconductive fine particles upon the reaction of the probe with the target substance. Specifically, the probe includes nucleic acids such as DNA and RNA and proteins such as an antigen and an antibody. Further, the probe to be used may be natural or artificial. Moreover, the probe is not necessarily consisted of one kind of material but may comprise other material(s) so long as the detection is not impeded.

In case of using a single-stranded DNA as the probe, it can be obtained by preparing a double-stranded DNA by a well-known method, suspending the double-stranded DNA in distilled water or the like, and heating the suspension to 100°C for around 10 minutes followed by

rapidly cooling the suspension on ice.

Furthermore, the "reaction" according to the invention includes not only a chemical reaction but also a physical interaction. An example of the reaction includes hybridization between DNAs when a single-stranded DNA is used as a probe and a DNA complementary to the single-stranded DNA is used as a target substance, and the binding of an antibody with an antigen when the antibody is used as a probe and the antigen used as a target substance and the like.

In case of using DNA as the probe, the length of the DNA to be used as the probe is at least 2 to 3000 bp, preferably 4 to 100 bp, and further preferably 10 to 12 bp.

When an antibody is used as the target substance, the antibody or antigen which can react with a target antigen or antibody can be mentioned as the probe. It is also preferable to activate the probe by means of a specific group.

When the gold nano-particles are used as electroconductive fine particles and DNA or antibody is used as the probe, the probe is preferably activated with a specific group such as SH group or NH_2 group.

In the above case, the site to be activated is preferably the site other than that to be involved in the reaction (for example, when the target substance is DNA, it is the site including the target sequence and when the target substance is an antigen, it is the site which binds to the antibody). The site to be activated is preferably an end of the probe, and more preferably both ends of the probe.

The site to be activated may include a part of a site to be involved in the reaction so long as the detection of the target substance is not impeded.

A method of activating the probe may appropriately be selected from well-known methods in accordance with the kinds of the probe and electroconductive fine particles to be used. For example, when DNA is used as the probe, the probe can be activated by treating the probe with thiols having SH group such as 1,10-decanedithiol and diamines having NH_2 group such as 1,10-diaminodecane.

When an antibody is used as the probe and gold nano-particles are used as the electroconductive fine particles, an activated site which can bind to the antibody is formed by treating with a compound such as a mercaptopropionic acid, which has an SH group that binds to the surface of the gold nano-particles and a carboxyl group at the terminal thereof, and with N-hydroxysuccinimide or 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride. Thus, the probe having NH_2 group can be fixed to the gold nano-particles or to the electrode via a peptide bond.

In the electric resistance type detecting sensor according to the second embodiment, a recess is formed on the surface of the substrate according to the first embodiment and the film of electroconductive fine particles modified with the probe is formed on the inner surface of the recess. The other constitution of the electric resistance type detecting sensor of the second embodiment can be the same as that of the first embodiment.

The "recess" according to the invention specifically includes a recess having the size and shape in which the reaction between the probe and the substance to be detected can be carried out and in which the film of electroconductive fine particles can be formed.

Accordingly, the shape of the recess may be a round or polygonal cylinder or cone. The shape of cone is especially preferable. In other words, the area of the bottom of the recess is preferably smaller than that of the opening thereof.

Further, the number of the recesses existing on the surface of one substrate can be appropriately selected in accordance with an application the sensor. For example, when the substrate is 1 to 3 cm^2 in size, the number of recesses is 100 to 3, 000, preferably 500 or more, 1,000 or more, 1,500 or more, and 2,000 or less, 2,500 or less.

Moreover, the electrodes are preferably provided so as to be in contact with the film of electroconductive fine particles within the respective recesses and so as not to be in direct contact with each other.

The site where the electrodes are formed is not limited to the surface of the substrate, but may be an inside, a shoulder part or a bottom part of the recess.

The method of forming the recess can be selected from well-known methods in accordance with a material of the substrate and the size or shape of the recess. In case of using a glass substrate or a plastic substrate, for example, the predetermined recess can be formed on the surface of the substrate by forming a mask having a predetermined shape of patterns on the surface of the substrate and

using a chemical etching agent or a laser beam.

A sheet having a portion of the recess may be applied to the substrate by lamination or the like to form the recess on the surface of the substrate.

The method of forming electrodes can be a well-known method. For example, a mask having the predetermined pattern is formed on the surface of the substrate after which a metal film is evaporated thereon to form the electrodes.

Either of the above process of forming the recess and the process of forming the electrodes can be carried out beforehand.

The film of the gold nano-particles of the invention may include the binder mentioned above.

As the third embodiment, an electric resistance type detecting sensor comprising a substrate having two or more fine recesses formed on the surface thereof; a film of electroconductive fine particles formed on an inner surface of the each recess; and first and second electrodes formed so as to be electrically connected to the film of electroconductive fine particles, wherein the film of electroconductive fine particles is modified with a probe can be mentioned.

On the surface of the substrate according to this embodiment, two or more fine recesses are formed.

The first and second electrodes of the electric resistance type detecting sensor of this embodiment can be connected through one or two or more multiplexer, preferably an analog multiplexer. The multiplexer functions as demultiplexer. The multiplexer contains an

element which has a function of switching the concerned sensor based on an external address signal. As the multiplexer, one having an input terminal, an output terminal and an address input terminal for inputting an address signal, for example, may be used. The address input terminal is preferably connected to a control part such as a microcomputer for outputting an address signal.

The first and second electrodes connected via one or two or more multiplexers are preferably connected further through an electrical property measuring device such as a voltage measuring device, current measuring device or resistance measuring device for measuring electric properties such as voltage between electrodes, current or resistance.

The first and second electrodes may be connected through a lock-in amplifier circuit instead of the electrical property measuring device. The electrical property measuring device may be connected to an output terminal of the lock-in amplifier circuit. In this case, detecting a synchronous component in periodical change in voltage by means of a lock-in amplifier can remove or reduce the noise property generated in the measuring environment, so that the sensitivity of an electric signal (voltage) can be improved.

The electrical property measuring device preferably comprises an output terminal for outputting electric current, voltage or the like according to the quantity of the measured electrical property. The output terminal is preferably connected to the control part. In case of using the lock-in amplifier circuit instead of the electrical property measuring device, the control part is preferably connected to the output

terminal of the lock-in amplifier circuit. The control part preferably comprises a storage part such as a memory so as to store an output from the electrical property measuring device.

The control part is preferably connected to an output device such as a monitor or a printer. It is preferable to be arranged to output the electrical property stored in the storage part to the output device.

The components of this embodiment other than those mentioned above can be the same as those of the first and second embodiment as mentioned above.

Moreover, in the fourth embodiment, an electric resistance type detecting sensor comprising a substrate having two or more fine recesses formed on the surface thereof; a film of electroconductive fine particles formed on an inner surface of each recess; and first and second electrodes formed so as to be electrically connected to the film of electroconductive fine particles, wherein the film of electroconductive fine particles is modified with a probe and connected to at least one of the electrodes and/or other electroconductive fine particles is used so that the each recesses are respectively bound to different probes to electrically detect and verify the target substance in a substance to be detected easily and in a short time.

On the surface of the substrate of this embodiment, two or more fine recesses are formed.

The first or second electrode corresponding to the respective recesses may be electrically connected with each other. In this case, having the structure similar to that of the third embodiment allows

carrying out the measurement for the substances to be detected introduced into respective recesses.

"The first electrodes are formed on the surface of the substrate" includes the case where the first electrodes formed on the surface of the substrate are covered with an insulating material or the like. Thus, the site where the first electrodes are formed is not limited to the surface of the substrate but may be an inner part or a shoulder part of the recess.

The second electrodes may be exposed to the back surface. In case that the second electrodes are exposed to the back surface, all or a part of the second electrode may be further covered with an insulating material and the like.

The second electrodes can be formed so that two or more grooves which do not cross each other and preferably extend in parallel are formed on the back surface of the substrate and the grooves are filled with a conductor such as platinum. The second electrodes may also be formed by forming a through hole on a first substrate and providing a second substrate, which has two or more electrodes not crossing each other and preferably extending in parallel, on the back surface of the first substrate.

Furthermore, it is preferable that the two or more recesses are arranged in matrix formed from plurality of rows and columns wherein the first electrode in respective rows and the second electrode in respective columns are electrically connected to each other, respectively.

The row and column of the matrix preferably cross perpendicular to each other, but may cross at a desired angle. The rows and columns

may be straight or curved.

Respective columns of the first electrodes and respective rows of the second electrodes can be connected to a multiplexer, respectively. When the address signal to be applied to the multiplexer is changed in order, the output of the sensor can be measured for the respective recesses arranged in matrix.

With regard to the multiplexer, the control part, the electrical property measuring device, the lock-in amplifier circuit, the output device and the like, those mentioned in the above can be applied.

Such a structure has an advantage such that it is space-saving and many measurements can readily be carried out.

The method of detecting a target substance using the electric resistance type detecting sensor according to the above-mentioned first to fourth embodiments is now described below.

According to the invention, the test sample is first applied to the film of electroconductive fine particles modified with the probe.

The term "Apply to the film of electroconductive fine particles" according to the invention means that the test sample is brought into contact with the film of electroconductive fine particles. Specifically, it ca be carried out by dropping the test sample onto the film of electroconductive fine particles, for example.

Further, the "test sample" according to the invention is one obtained by treating the substance to be detected for the presence of the target substance so as not to impede the measurement. Specifically, it includes, for example, the one which is obtained by diluting a substance

to be detected with an appropriate solvent so as to obtain an appropriate concentration for the detection, or the one which is obtained by treating an obtained double-stranded DNA into a single-stranded DNA suitable for the detection.

The quantity of the test sample is not specifically limited so long as the test sample can contact with at least the film of electroconductive fine particles in the recess on the substrate.

Moreover, a condition for the reaction between the probe and the test sample can be appropriately selected in accordance with the probe or the test sample to be used.

The presence of the target substance can be detected and verified by measuring an electric resistance value among the obtained film of electroconductive fine particles and measuring an electric resistance value of the films before and after applying the test sample by using a well-known method.

Generally, the electric current flows through between electrodes via the film of electroconductive fine particles. When the test sample contains the target substance and the target substance reacts with the probes, the electric current flows via probes, thus allowing the detection and verification of the presence of the target substance.

Besides the electric resistance type detecting sensor according to the above first to fourth embodiments, the presence of the target substance which reacts with the probe can be detected and verified by, as the fifth embodiment, modifying the film of electroconductive fine particles formed on the surface of the electrically insulated substrate

with the probe and applying the test sample containing the substance to be detected to the thus modified film, and measuring an electric resistance value between two points of the obtained film of electroconductive fine particles.

Furthermore, in accordance with the detecting method in the sixth embodiment, the presence of the target substance which reacts with the probe can be detected and verified by preparing the test sample containing the substance to be detected and the probe in advance, applying the sample onto the film of electroconductive fine particles formed on the surface of the electrically insulated substrate, and measuring an electric resistance value between two points on the obtained film of electroconductive fine particles.

The detecting method of this embodiment is different from the above detecting methods in that the previously prepared test sample containing the substance to be detected and the probe is applied to the film of electroconductive fine particles on the substrate, which is not modified with the probe, to measure the electric resistance value among the film.

Therefore, the above-mentioned detecting method does not require a step for modifying the film of electroconductive fine particles with the probe.

The above term "preparing the test sample" includes that the probe and the test sample are exposed to the condition under which the target substance and the probe can react.

Specifically, when the single-stranded DNA of around 12 bp is

used as the probe, the test sample can be prepared by leaving the probe and the test sample to be detected for 30 minutes at room temperature.

For components other than the above, those mentioned above can be applied.

EXAMPLE 1

In Example 1, selectivity of the present electric resistance type detecting sensor is examined.

In this Example, 200 ml of an aqueous solution containing 6 ml of 1% hydrogen tetrachloroauric acid (III) tetrahydrate (Wako Pure Chemical Industries, Ltd.) aqueous solution and 10 ml of 3% citric acid (Katayama Chemical) aqueous solution was agitated at 80°C for 20 minutes to prepare a gold colloidal solution. Then, a "comb-shaped platinum electrode" (manufactured by BAS Inc.), which was obtained by evaporating platinum on a glass substrate (1 cm × 1 cm) so that a gap between electrodes was 5 μm, was immersed in 1,10-decanedithiol/ethanol solution, followed by in the gold colloidal solution containing gold nano-particles to form a film of the gold nano-particles on and between the electrodes. The thus-obtained film was added dropwise with 5 μl of a solution of 100 μM DNA (SEQ ID NO:1: 5'–TCTCAACTCGTA-3') whose 5'-terminal is activated with SH group, as a probe, and left to stand for 30 minutes to modify the film of the gold nano-particles between the electrodes with the probe.

The obtained film was dropped with 1 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, 1M NaCl) to moisturize the film of the gold

nano-particles and left to stand until an electric resistance value of the film became stable. A TE buffer solution containing 100 μM of DNA, which has the sequence of SEQ ID NO:2 to 5, was prepared as the test sample and 5 μl of the prepared solution was added dropwise onto the above-obtained film.

Fig. 1 shows the change in resistance value of the film before and after the detection when the DNA having the sequence of SEQ ID NO:1 was used as the probe and the DNA having the sequence of SEQ ID NO:2 (a single-stranded DNA in which 2:1 bp is complementary), the DNA having the sequence of SEQ ID NO:3 (a single-stranded DNA in which 3:8 bp is complementary), the DNA having the sequence of SEQ ID NO:4 (a single-stranded DNA in which 4:11 bp is complementary) and the DNA having the sequence of SEQ ID NO:5 (a single-stranded DNA in which 5:12 bp is complementary) were used as the test sample, respectively. As a result, when the test sample was dropped on the film of the gold nano-particles modified with the DNA, the electric resistance of the film was reduced and the resistance value of the film became stable after around 1 minute. The variation in electric resistance values before and after the detection was the largest (5.16×10^{-2} Ωcm) in case of using the DNA of SEQ ID NO:5 as the test sample (5). On the other hand, the variation in electric resistance value of the film was around 2.40×10^{-2} Ωcm (4), 1.44×10^{-2} Ωcm (3) and 1.39×10^{-2} Ωcm (2) in case of using the DNA other than the DNA of SEQ ID NO:5 was used as the test sample. Thus, the variation in electric resistance values of the film before and after the detection was 1.01×10^{-2} Ωcm/base for (4)

and (2), while it was 2.76×10^{-2} Ωcm/base between (4) and (5). The most significant variation was found between (4) and (5). This result means that by using the electric resistance type detecting sensor of the invention, efficient detection can be carried out even with the one which has a difference of 1 bp. from the target DNA. This indicates that the electric resistance type detecting sensor of the invention is superior in selectivity.

The film of the gold nano-particles was modified with DNA having the sequence of SEQ ID NO:1 as the probe as described above, and then, 1 μl of the TE buffer solution was dropped on the obtained film of the gold nano-particles to moisture the film evenly. The film of the gold nano-particles was left to stand until the electric resistance value thereof became stable. After about 100 seconds from the stabilization, 5 μl of the TE buffer solution containing DNA having the sequence of SEQ ID NO:5 was dropped on the film of the gold nano-particles. The film of the gold nano-particles was again left to stand until the electric resistance value of the film became stable. Then, 10 μl of an enzyme DNase I (Wako Pure Chemical Industries, Ltd., 10 mg/ml) was dropped on the film and the film was left to stand for about one hour at room temperature so that the DNA bound to the film of the gold nano-particles was degraded, and thus, the film was returned to the state before modification with the probe. Fig. 2 shows electric resistance values of the film before and after the degradation of DNA existing on the film of the gold nano-particles modified with DNA (probe) with the DNase. As a

result, the electric resistance value of the film of the gold nano-particles after degradation of the DNA with which the film was modified were almost the same as that of the film of the gold nano-particles before the modification with DNA (624.36Ω). This means that the electric resistance type detecting sensor of the present invention can be repeatedly used.

EXAMPLE 2

The electric resistance values of the film was measured by the method similar to that of Example 1 except that the DNA having the sequence of SEQ ID NO: 5 was used as the test sample and the DNA having the sequence of SEQ ID NO:1 both ends (3' and 5') of which being thiol-derivatized (manufactured by NISSHINBO) was used as the probe.

As shown in Fig. 3, the variation in electric resistance of the film before and after the detection increased 2.9 times higher than that obtained when the only one end (5') of the DNA was thiol-derivatized was used as the probe (Example 1 (5)) (Example 1: 0.30Ω, Example 2: 0.87Ω). This indicates that increase in number of modified sites of the probe causes increase in sensitivity of the detection.

EXAMPLE 3

A gold colloidal solution (1.5 ml) containing gold nano-particles was mixed with 5 μl of an aqueous solution containing 50 μM of DNA having the sequence of SEQ ID NO:1 both ends (3' and 5') of which being thiol-derivatized, and the mixture was left to stand for 30 minutes to

modify the gold nano-particles with the probe DNA (SEQ ID NO:1). After the centrifugation (6000 rpm, 10 minutes) of the solution, the solution was re-dispersed with 1.5 ml of water. The above operation was repeated twice, and at the end, the solution was re-dispersed with 0.5 ml of water. The obtained solution (30 μl) was dropped onto the comb-shaped platinum electrode (BAS INC.) to form a film of gold nano-particles without a binder on or between the electrodes. The electric resistance value was measured according to the method similar to that of Example 2.

As a result, variation in the electric resistance value of the film before and after the detection was 0.57Ω, as shown in Fig. 4. This shows that the present electric resistance type detecting sensor can detect a target substance even when the film of electroconductive fine particles does not include a binder.

EXAMPLE 4

The same glass substrate and comb-shaped platinum electrode as those used in Example 1 were used. A TE buffer solution (5 μl) containing 100 μM of DNA, as the probe, having the sequence of SEQ ID NO:1 5' end of which being thiol-derivatized was mixed with 5 μl of the TE buffer solution containing 100 μM of DNA having the sequence of SEQ ID NO:5 as the test sample in a micro-tube (TreffLab, manufactured by Treff AG Company, Switzerland) in advance for the preparation. The electrodes were immersed in 1,10-decanedithiol/ethanol solution, and then, immersed in the gold colloidal solution to form the film of the gold

nano-particles on and between the electrodes. The film of the gold nano-particles was moisturized with 1 μl of TE buffer solution, the above prepared solution was dropped onto the film of the gold nano-particles and the electric resistance values of the film were measured before and after the dripping. The result is shown in Fig. 5.

As a result, the electric resistance value of the film before and after the detection increased by 2.7 fold (0.81Ω) compared to that obtained when DNA only 5' end of which being thiol-derivatized was used as the probe in Example 1 (0.30 Ω). This shows that the detecting method according to the sixth embodiment of the invention is effective.

EXAMPLE 5

This Example shows the case when an antibody was used as the probe.

The same glass substrate and comb-shaped platinum electrode as those used in Example 1 were used. These were immersed in 1,10-decanedithiol/ethanol solution, and then, immersed in the gold colloidal solution to form the film of gold nano-particles on and between electrodes. The electrodes on and between which the film of a gold nano-particle being formed were immersed in 10 mM mercaptopropionic acid (TOKYO CHEMICAL) solution in ethanol for 30 minutes to modify the film of the gold nano-particles with mercaptopropionic acid. The thus-obtained film was rinsed with ultra pure water and washed by ultrasonication in ethanol for 5 minutes. Then, the film of the gold nano-particles was rinsed with ultra pure water and dried. The film of

the gold nano-particles was then contacted with 20 μl of 100 mg/μl N-hydroxysuccinimide (Wako Pure Chemical Industries, Ltd.) solution, followed by with 20 μl of 100 mg/μl 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (WSC) (DOJINDO) solution and left to stand at room temperature.

Then, the obtained film of the gold nano-particles was rinsed with ultra pure water, and washed with 0.1 M Tris-hydrochloride buffer solution (pH8), followed by dripping thereon 10 μl of 0.1 M Tris-hydrochloride buffer solution to leave the film to stand at room temperature for 100 seconds. On the obtained film was dropped with 10 μl of rabbit-anti-mouse IgG (Wako Pure Chemical Industries, Ltd.) antibody solution diluted 100 times with 0.1 M Tris-hydrochloride buffer solution, and the film was left to stand at room temperature for 30 minutes.

Then, the obtained film was rinsed with Tris-hydrochloride buffer solution, and 10 μl of 0.1 M Tris-hydrochloride buffer solution followed by 20 μl of ethanolamine (Wako Pure Chemical Industries, Ltd.) solution were dropped thereon. The film was left to stand at room temperature for 1 hour to mask the activated sites on which the antibodies were not fixed.

The film of the gold nano-particles modified with the antibody was washed with ultra pure water followed by with Tris-hydrochloride buffer solution. The film of the gold nano-particles was then exposed to 10 μl of Tris-hydrochloride buffer solution.

After the film was left to stand for 100 seconds, 10 μl of an

antigen solution of 100 μg of mouse IgG (Upstate BioTechnology Company)/ 1 μL of Tris-hydrochloride buffer solution was dropped on the obtained film. Immediately after the dripping, the electric resistance value of the film was measured and the result is shown in Fig. 6.

The result in Fig. 6 shows that the electric resistance type detecting sensor of the invention can be applied to the detection of an antigen.

EXAMPLE 6

Fig. 7 shows the electric resistance type detecting sensor 51 in accordance with Example 6 of the invention. The electric resistance type detecting sensor 51 in accordance with Example 2 of the invention comprises two or more recesses 53 formed on the surface of the substrate 54 and the film 57 of the gold nano-particles is formed on the inner surface of each recess 53. First and second electrodes 55 and 56 are formed so as to be electrically connected to the film 57 of the gold nano-particles of each recess 53. The electric resistance type detecting sensor 51 is manufactured according to the following method.

Forming and Cleaning Electrodes

First, two or more platinum electrodes extending parallel to each other are formed on the substrate. The platinum electrodes can be formed by evaporating platinum with the sites other than which platinum electrodes will be formed being masked. Then, as shown in Fig. 7(a), recesses are formed one by one at the center of the respective

electrodes so as to dividing the platinum electrode into two. The recess thus divides the platinum electrode to form the first and second electrodes. After forming the recesses, the platinum electrodes are cleaned in the following method.

The above platinum electrodes are washed by repeating a sweep 50 times in a range of -0.25 to +1.3 V at the sweeping velocity of 200 mV/s in 0.1M of H_2SO_4 with Ag/AgCl being used as a reference electrode and with a platinum coil (manufactured by NILAKO Company) being used as an antielectrode. Electrochemical washing is performed with a potentiostat (manufactured by SEIKO EG & G Company, 263A-1).

Forming of Film of Gold Nano-Particles

The method of forming the film of the gold nano-particles is described.

An aqueous solution (200 ml) containing 6 ml of 1% hydrogen tetrachloroauric acid (III) tetrahydrate (Wako Pure Chemical Industries, Ltd.) aqueous solution and 10 ml of 3% citric acid (Katayama Chemical) aqueous solution was agitated at 80°C for 20 minutes to prepare a gold colloidal solution.

Then, an ethanol solution containing 5 mM of 1,10-decanedithiol is injected in the recesses and the recesses are rinsed with ethanol after the ethanol evaporates. The gold colloidal solution is then injected to the recesses so that the films of the gold-nano particles are formed on the surface of the recesses.

By forming the film of the gold nano-particles as described above, the first and second electrodes 55 and 56 are electrically connected with

the film 57 of the gold nano-particles of each recess 53.

Modification with DNA Probes

DNA 5' end of which being thiol-derivatized (manufactured by NISSHINBO) was used as the probe. The recesses (the volume of the recess is 1 mm^3) are filled with 1 μl of TE buffer solution (10 mM Tris-HCl, 1mM EDTA, 1M NaCl) containing 100 μM of the above-mentioned DNA and are left to stand for 30 minutes. By this, the gold nano-particles are modified with the probe DNA.

Then, the surface of the gold nano-particles is washed with the TE buffer solution in order to remove an excess thiol-derivatized DNA. Further, the surface of the gold nano-particles is moisturized with 1 μl of the TE buffer solution in order to remove the influence of the surface resistance.

Peripheral Devices

Now, peripheral devices and the like to be connected to the electric resistance type detecting sensor 51 are described.

As shown in Fig. 7, the first electrodes 55 corresponding to respective recesses 53 is electrically connected with an input terminal 61 of a multiplexer 60. An output terminal 62 of the multiplexer 60 and the second electrodes 56 corresponding to respective recesses 53 are electrically connected through an electric resistance measuring instrument 63. The electric resistance measuring instrument 63 outputs from its output terminal 64 the voltage corresponding to the measured electric resistance. A microcomputer 65 is connected to an address input terminal 66 of the multiplexer 60 and the output terminal

64 of the electric resistance measuring instrument 63. The multiplexer 60 here functions as a demultiplexer, and therefore, the plurality of the output from the respective recesses 53 are inputted to the input terminal 61 and outputted from the single output terminal 62.

The microcomputer 65 changes the output addresses one by one to store outputs of the electric resistance measuring device 63 for the respective recesses 53. The microcomputer 65 is connected to an output device 67 such as a printer and a monitor and is arranged to output the stored data to the output device 67. Such a structure allows many target DNAs to be simply detected at one time.

Detection of Target DNA

Now, the method of detecting the target DNA by means of the electric resistance type detecting sensor 51 in accordance with the invention and the peripheral devices thereof is described.

First, 50 μl of TE buffer solution containing 100 μM of the test sample is evenly dropped on the surface of the gold nano-particles, which is prepared in advance, in the recess 53 of the electric resistance type detecting sensor and the recess is left to stand for 3 minutes. Then, a digital multi-meter (manufactured by HEWLETT PACKARD Company, 34401A Model) 63 is used to measure the electric resistance at the both ends of the electrodes at 22 ± 1°C.

EXAMPLE 7

Fig. 8 is a block diagram showing the structure of the electric resistance type detecting sensor in accordance with Example 7. In the

electric resistance type detecting sensor in accordance with Example 7, the output terminal 62 of the multiplexer 60 and the second electrodes 56 corresponding to the respective recesses 53 are electrically connected through a lock-in amplifier circuit 68. An output terminal 69 of the lock-in amplifier circuit 68 is connected to the microcomputer 65. Other structure and a method of forming the gold nano-particles or the like are same as those of Example 6.

Fig. 9 is a block diagram showing the structure of the lock-in amplifier circuit 68 in accordance with Example 7. A denotes an adder, B denotes the electric resistance type detecting sensor shown by a dotted line in Fig. 3, C denotes a current-voltage converter, D denotes a lock-in amplifier, E denotes a bias voltage and F denotes a synchronous signal. By selectively detecting an output component synchronous to the signal F by means of the lock-in amplifier, noise generated in the measurement environment can be removed or reduced.

EXAMPLE 8

Fig. 10 shows an electric resistance type detecting sensor 71 in accordance with Example 7 of the invention, which comprises a plurality of the above-mentioned electric resistance type detecting sensors on the substrate. The electric resistance type detecting sensor 71 comprises two or more recesses 73 arranged in a matrix consisting of two or more rows X and columns Y.

Further, a film 77 of gold nano-particles is formed on the inner surface of each recess 73. First and second electrodes 75 and 76 are

formed so as to be electrically connected to the film 77 of the gold nano-particles of the respective recesses 73. The electrode 75 may be formed in the shape of ring or the shape similar to ring on the surface of the recess 73 of a substrate 74.

The first electrodes 75 are formed on the surface of the substrate 74. The second electrodes 76 are formed in the recesses 73 and exposed to the back surface of the substrate 74. The first electrode 75 in each row X and the second electrode 76 in each column Y are electrically connected to each other.

Forming Electrode and Film of Gold Nano-Particles

Now, a method of forming the above-mentioned electrodes 75 and 76 and the film 77 of the gold nano-particles electrically connected to the electrodes 75 and 76 is described.

First, two or more grooves extending parallel to each other are formed on the back surface of the substrate and the second electrodes 76 are formed from platinum so as to fill in the grooves.

Second, the first electrodes 75 are formed in the shape as shown in Fig. 10(a).

Then, two or more recesses 73 arranged in a matrix are formed in the surface of the substrate 74 so as to be opposed to the second electrodes 76. The recess 73 is formed with the depth that the second electrode 76 is exposed to the surface side of the substrate 74.

Finally, the film 77 of the gold nano-particles is formed on the inner surface of the recess 73 by the method similar to the one used in Example 6 to complete the formation of the electrodes and the film of the

gold nano-particles.

Modification with DNA Probe

Then, the film 77 of the gold nano-particles is modified with the DNA probe by the method similar to the one used in Example 6.

Peripheral Devices

Now, peripheral devices and the like to be connected to the electric resistance type detecting sensor 71 are described.

As shown in Fig. 10, the respective columns Y of the first electrodes 75 and the respective rows X of the second electrodes 76 are respectively connected to the input terminals 82 and 83 of the multiplexers 80 and 81, respectively. The output terminals 84 and 85 of the respective multiplexers 80 and 81 are electrically connected to each other through an electric resistance measuring instrument 86. For the electric resistance measuring instrument 86, may use the lock-in amplifier circuit 68 shown in Fig. 9. The electric resistance measuring instrument 86 outputs from its output terminal 87 the voltage corresponding to the electric resistance measured. A microcomputer 88 is connected to address input terminals 89 and 90 of the respective multiplexers 80 and 81 and to the output terminal 87 of the electric resistance measuring device 86.

Moreover, the microcomputer 88 changes the output addresses corresponding to the respective multiplexers 80 and 81 one by one to output the same, scans the recess 73 arranged in the two-dimensional array and stores outputs of the electric resistance measuring device 86 for the respective recesses 73. The microcomputer 88 is connected to

an output device 91 such as a printer and a monitor and is arranged to output the stored data to the output device 91.

Such a structure allows more target DNAs to be detected at one time. In addition, the structure in which the first electrodes are provided on the surface of the substrate while the second electrodes are provided on the back surface of the substrate allows the sensor to be provided at a high density, so that space-saving of the device can be also achieved.

Detection of Target DNA

Detection of target DNA can be carried out with the electric resistance type detecting sensor 71 of Example 9 and its peripheral devices by using the method similar to the one used in Example 6.

INDUSTRIAL APPLICABILITY

Using the electric resistance type detecting sensor in accordance with the invention allows the presence of a target substance to be electrically detected and verified more easily, rapidly, at a lower cost, compactly and with better accuracy than the conventional sensors without using a specific reagent such as a fluorescent material or a complicated device.

Furthermore, the electric resistance type detecting sensor in accordance with the invention can be used repeatedly, more easily, rapidly and at a lower cost than the conventional sensors.

Moreover, forming the recess on the substrate, forming in the recess the film of electroconductive fine particles modified with the probe

and allowing the test sample to be reacted with the probe in the recess allow a space required to the reaction to be reduced.

In addition, forming two or more recesses on the same substrate allows many kinds of test samples and/or target substances to be electrically detected and verified at one time.

Further, using the gold nano-particles as the electroconductive fine particles and using DNA or an antibody activated with SH group or NH_2 group as the probe allow the target substance to be detected and verified more accurately.

Moreover, activating one end of the probe allows the target substance to be detected and verified more accurately.

Furthermore, activating both ends of the probe allows the target substance to be detected and verified more accurately.

In addition, forming the recess into the shape of cone allows detection to be more efficiently carried out.

Moreover, using the above-mentioned electric resistance type detecting method in accordance with the invention allows a target substance to be electrically detected and verified more easily, rapidly, at a lower cost, compactly and with better accuracy than the conventional methods without using a specific reagent such as a fluorescent material or a complicated device.

Further, using the electric resistance type detecting method in accordance with the invention allows a target substance to be detected and verified repeatedly, more easily, speedy and at a lower cost than the conventional methods.